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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 11 AUGUST 1953 No. 8

PANTOTHENIC ACID IN NUTRITION

Because of the fact that *single* vitamin deficiencies are seldom seen by clinicians, and because the B-complex vitamins are so intimately associated in both their natural distribution and biochemical action, it seems reasonable to consider those vitamins that have a well-demonstrated biochemical role in other mammals as "required," tentatively—even though evidence for this in the form of clear-cut signs and symptoms is scanty.

If one uses the above guides to develop a more liberal standard for the establishment of a vitamin in the "required" class, then pantothenic acid can be considered with some confidence as a vitamin needed in human nutrition.

The existence of a growth substance for yeast called "bios" led to our first knowledge of pantothenic acid. In 1940 it was isolated in pure form and its chemical structure was established (R. J. Williams and R. T. Major, *Science* **91,** *246 (1940)*). The fact that pantothenic acid was a growth factor for numerous microorganisms provided a tool for evaluating its distribution in natural products. It was soon found that assay values obtained with chicks were considerably higher than those obtained by microbiologic methods. This discrepancy can now be explained by the fact that the vitamin exists primarily in bound forms which are not measured by the usual microbiologic procedures. More complete enzymatic liberation of pantothenic acid followed by its microbiologic determination confirmed the fact that earlier published values of its occurrence were far too low (J. B. Neilands and F. M. Strong, *Arch. Biochem.* **19,** *287 (1948)*).

On the basis of these newer assay technics, it is quite evident that not only is pantothenic acid very widely distributed in nature, but it is present in natural materials in significant amounts and considerably in excess of most of the other B-vitamins. This certainly suggests a vital metabolic function for pantothenic acid.

The discovery of this metabolic or biochemical function was not long in coming. In 1945, F. Lipmann (*J. Biol. Chem.* **160,** *173 (1945)*) reported that a cell-free preparation of pigeon liver, when allowed to respire, would acetylate considerable amounts of sulfanilamide. Anaerobically, little acetylation occurred unless ATP was added. When the tissue was allowed to autolyze, no sulfanilamide was acetylated aerobically, although such an inactivated preparation could be reactivated by additions of boiled liver juice. This suggested that a heat-stable co-factor was involved and that the acetylation reaction probably consisted of an apoenzyme-coenzyme complex with ATP as the energy donor.

In 1947 F. Lipmann *et al.* (*J. Biol. Chem.* **167,** *869 (1947)*) were able to concentrate this factor, which they called coenzyme A (A for acetylation), and after rigorous analysis found that pantothenic acid in a bound form was indeed the active principle. Numerous studies have shown this enzyme to be important not only in acetylation reactions involving sulfanilamide, choline, para-aminobenzoic acid and histamine, but also to be intimately concerned in the vital metabolic reactions involving acetate (to acetoacetate), and oxaloacetate (to citrate). S. Korkes, A. del Campillo, I. C. Gunsalus, and S. Ochoa (*Ibid.* **193,** *721 (1951)*) have shown that coenzyme A functions in the condensation of pyruvate with oxaloacetate to form citrate. The formation of the compound, acetyl-co A ("active acetate"), is believed to represent an essential step in the oxidative metabolism of carbohydrate and

presumably fatty acids and amino acids. Pantothenic acid is therefore unquestionably one of the essential components of the key reactions involving the cellular oxidation of food materials.

Other bound forms of pantothenic acid have been demonstrated (*Nutrition Reviews* **7,** *348 (1949)*; **9,** *78, 238 (1951)*). A molecule somewhat larger than co A, called pantothenic acid conjugate (PAC), one of the bound forms of pantothenic acid, has been studied. Although PAC does not have co A activity, similar enzymatic treatment is needed for the liberation of its pantothenic acid. PAC, as well as co A and the enzyme-liberated fragments of co A all stimulate the growth of the microorganism, *Acetobacter suboxidans*, which therefore provides a means by which the total bound pantothenic acid content of tissues can be measured.

Pantothenic acid has also been associated with the bacterial growth factor, the *Lactobacillus bulgaricus* factor (LBF). The sulfur-containing pantothenic acid compounds pantetheine (—SH) and pantethine (—S—S—) appear to be identical with the LBF, and provide important clues to the structure of coenzyme A itself.

How is the co A activity of tissues affected by a deficiency of pantothenic acid? Is a biochemical lesion possible? T. R. Riggs and D. M. Hegsted (*J. Biol. Chem.* **172,** *539 (1948)*) have reported that rats on a pantothenic acid-free diet show a decreased ability to acetylate para-aminobenzoic acid, as determined by the levels of acetyl compound in urine after a test dose of the free acid.

R. E. Olson and N. O. Kaplan (*J. Biol. Chem.* **175,** *515 (1948)*) have studied the effect of dietary pantothenic acid deficiency on the co A content and the pyruvate utilization of rat and duck tissues. Rats placed on a diet low in pantothenic acid maintain normal levels of co A in their tissues for two to three weeks, but show a gradual depletion to a level of 25 to 40 per cent of normal at six weeks. Ducklings, on the other hand, show a rapid loss of the co A content of their tissues, reaching a minimal level of 40 per cent of normal in five days. Liver slices from both species of the deficient animals exhibit a decreased ability to utilize pyruvic acid, which accompanies the low co A content of this tissue.

Although it is true that some of the acetylation reactions serve a "detoxication" function, there is evidence that acetylation is a more fundamental process. The ubiquitous occurrence of co A argues for basic metabolic functions. The importance of the acetate unit in the biosynthesis of such complex structures as fatty acids, the ring structure of cholesterol and other sterols, and of hemin, seems to imply acetylation.

Beyond the biochemical evidence of pantothenic acid deficiency, what of the symptoms and pathology that may be seen? The chief lesions of pantothenic acid deficiency in experimental animals appear to involve the nervous system, the adrenal cortex, and the skin.

In swine, a marked evidence of pantothenic acid deficiency involves the nervous system, which results in locomotor disturbances (R. H. Follis and M. M. Wintrobe, *J. Exp. Med.* **81,** *539 (1945)*). The chief microscopic lesions appear to be a chromatolysis of isolated cells of the dorsal root ganglia. This is followed by a demyelinizing process, evident in the brachial and sciatic nerves. Because of these findings it has been suggested that pantothenic acid plays a role in the etiology of human deficiency polyneuritis. Little evidence is available to support this view.

One of the most interesting effects of pantothenic acid deficiency is the marked involvement of the adrenals (*Nutrition Reviews* **10,** *221 (1952)*). Grossly, the glands are hypertrophied, pinkish, sometimes with obvious gross signs of isolated hemorrhages into the parenchyma. Various types of pathologic change are evident in such adrenal glands. Most of these changes in-

volve the inner zones of the adrenal cortex. Loss of lipid and ketosteroids from the inner zones with little change in the zona glomerulosa is seen. An investigation of the functional integrity of adrenal cortex in the pantothenic acid-deficient rat (W. A. Krehl, R. W. Winters, and R. B. Schultz, *Nutrition Symposium Series* **5**, *39* (*1952*), *National Vitamin Foundation, Inc.*) reveals that the deficient animal shows an insufficiency of adrenal cortical function in those phases of its activity usually attributed to the C-11 oxysteroid hormone (cortisone). This is evidenced by a lack of eosinopenia and lymphocytopenia in response to epinephrine administration, an increase in sensitivity to insulin, an inability to excrete normal amounts of glucose and nitrogen after phlorhizinization, and a reduction in the quantity of hepatic glycogen deposited in the fasting state, when compared to control animals. That these effects were mediated through the adrenal itself and not materially influenced by the pantothenic acid-deficient peripheral tissues is indicated by the refractoriness of these animals to ACTH administration and the correction of the above defects with cortisone.

Histologic examination of the adrenals of untreated and ACTH- and cortisone-treated animals indicates that the adrenal damage of pantothenic acid deficiency may be greatly magnified by treatment with ACTH. An exogenous supply of cortisone markedly inhibits the development of the characteristic adrenal lesion, however. Further, cholesterol demonstrable in the adrenal cortex of deficient animals may be reduced to the vanishing point by ACTH treatment, while cortisone-treated deficient rats have nearly normal levels of adrenal cholesterol.

E. P. Ralli (*Nutrition Symposium Series* **5**, *78* (*1952*), *National Vitamin Foundation, Inc.*) has found further evidence for the importance of pantothenic acid in stress conditions imposed on human subjects. "The administration of calcium pantothenate in large doses (10 g. per day for six weeks) influenced some of the blood and urinary findings elicited by immersing young men in cold water. The significant changes observed after the stress, following therapy with pantothenate, were elevated whole blood ascorbic acid; less of an eosinopenia, with an increase in the absolute number of eosinophils; an increased uric acid/creatinine ratio; significantly less variation in the total white blood cell count, neutrophils and lymphocytes from the prestress (control) values; a lower level of serum chloride, expressed either in milliequivalents per liter or in milliequivalents per 100 g. of serum water and a lower serum level of total cholesterol and cholesterol fractions." It is further stated that, "It has not seemed an unreasonable premise to suggest that the cellular response to stress may be influenced by the administration of substances which are important to intracellular reactions." Whether or not these very large doses of the vitamin are actually needed can only be resolved by further work.

Yet another evidence of pantothenic acid deficiency is a greying of fur and feathers. This may be complicated by concomitant deficiencies of folacin and biotin. Adrenalectomy, however, stimulates pigmentation in the presence or absence of pantothenic acid deficiency. The mechanism here is little understood.

The premature and overenthusiastic use of pantothenic acid in the futile attempts to cure greying of the hair in human subjects is the type of event which helps to create an overly and not entirely unjustified conservative attitude on the part of responsible government agencies and many nutritionists.

Recent observations have indicated a possible relationship between the "burning feet syndrome" as seen particularly in Japanese prisoners-of-war and pantothenic acid deficiency. This syndrome appears to be a distinct entity and apparently is of nutritional etiology; in fact, it has been called "nutritional melalgia" (M. Glusman,

Am. J. Med. **3,** *211 (1947)*; S. Vernon, *J. Am. Med. Assn.* **143,** *799 (1950)*).

Burning feet as a clinical symptom is not a new entity. In its milder forms, it is characterized by an insidious onset of bilateral paresthesias affecting the lower extremities. Later, the patients complain of severe shooting pains which appear to be aggravated by exertion or warmth and alleviated by cold. In the most advanced forms, the patients are extremely sensitive to such minor stimuli as floor vibrations; the pains now are definitely established above the knee and even the upper extremities may be affected. As usually observed, the syndrome does not demonstrate prominent muscle wasting, paralysis, or areflexia.

Numerous investigators have noted that this nutritional disease does not respond to thiamine or niacin. Although pantothenic acid has been used with dramatic results, clinicians who have had the opportunity of first-hand observations of this disease have noted that whenever the general quality of the dietary is increased there is a general alleviation of the painful feet and have suggested that one of the lesser known nutritional factors is involved, most likely pantothenic acid.

If we consider pantothenic acid to be a required vitamin, what estimates can be made of the amount that should be recommended for a daily allowance? Studies on the urinary excretion of this vitamin by normal subjects indicate considerable variation, from 2.5 to 9.6 mg. per day, with an average excretion of 5.7 mg. (J. Fitzpatrick and S. L. Tompsett, *J. Clin. Path.* **3,** *69 (1950)*). An analysis of several other reports on this subject indicate excretion values somewhat lower than the average value indicated above and in a somewhat lower range. The reason why pantothenic acid deficiency in human subjects is not readily seen can be discerned from the results of V. Schmidt (*Z. f. Vitamin-, Hormon- u. fermentforsch.* **4,** *322 (1951)*), who found after an analysis of Danish foods and Danish diets for pantothenic acid that the average daily intake of pantothenic acid was in the range of 10 to 20 mg. per day, or about 5 mg. per 1000 calories.

If one considers the normal excretion levels of pantothenic acid, the increased excretion following a small test dose of the vitamin and the average intake figures quoted above, it would not seem unreasonable to set the recommended allowance at a level of 10 to 15 mg. per day—or about the same as that recommended for niacin. The fact that clinical signs of pantothenic acid deficiency are apparently seldom in evidence indicates that the 10 to 20 mg. amount supplied in average daily diets is adequate.

H. Pollack and S. L. Halpern ("Therapeutic Nutrition," *Publication* **234,** *National Academy of Sciences—National Research Council, Washington, D. C. (1952)*) say that pantothenic acid should be included in all vitamin supplements until evidence is produced in the future that it is not needed. However, one might just as well say the opposite, and perhaps more logically, since ill-health in man known to be related to a lack of pantothenic acid in the diet is rare indeed. Pollack and Halpern state further, "It is recommended that in cases of severe illness, injury or burns, and in cases of known nutritional depletion 20 mg. of calcium pantothenate be administered daily until convalescence is well established and the patient again is eating a good diet." There is as yet, however, little objective evidence to support such a statement and it would seem that in the case of severe trauma evidence on this point should not be difficult to obtain.

It remains for the future to show that ill-health in man is occasioned by lack of pantothenic acid in the diet and if extra amounts of it have useful roles to play in preventive and curative medicine and in improved health.

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ADULT STANDARDS OF BODY WEIGHT IN THE UNITED STATES

Standards of body weight, in spite of all their limitations, remain the point of departure in the evaluation of nutritional status. A review of some recent publications concerned with this topic may be useful.

Valuable information on the body size of young American men and women has been compiled by the Anthropology Unit of the Quartermaster Climatic Research Laboratory, Lawrence, Mass., as a by-product of research on bodily dimensions of Army personnel measured in relation to clothing sizing and spatial requirements.

The methodology of collecting anthropometric data was described in detail by F. E. Randall and M. J. Baer ("Survey of Body Size of Army Personnel," *Office Quartermaster General, Environmental Protection Branch, Report No. 122, Revised (1951)*). The instruments used (anthropometer, a spreading and a sliding caliper, and steel tape) and the actual technics utilized in measuring the subjects are described verbally and pictorially. There is a diagram of the skeletal parts and bony reference points, and a glossary of anatomic terms.

The publication is a useful addition to available manuals dealing wholly (T. D. Stewart, "Hrdlička's Practical Anthropometry," *4th ed., Wistar Institute, Philadelphia (1952)*), or partly (M. F. A. Montagu, "An Introduction to Physical Anthropology," *2d ed., C. C. Thomas, Springfield, Ill. (1951)*), with the methods of measurement in physical anthropology. However, only a negligible fragment of the classic anthropologic measurements is of direct interest to the nutritionist (weight, stature, some diameters and circumferences). The measurement of skinfold thickness is not considered in any of the three publications dealing with anthropometric methods.

In the past, chest circumference has been used by several authors as a criterion of "body build," in addition to height. E. H. Munroe ("Preparation of Anthropometric Nomographs," *Report No. 186 (1952)*) used stature and chest circumference as the basic variables, and weight as one of the numerous dependent variables important for the designers of clothing and other personal equipment. At a height of 68.5 inches, the average height of Selective Service registrants, the mean weight of U. S. white Army males varies from about 130 pounds for individuals with small chest (circumference of 32 inches), through 153 pounds for medium chest (36 inches) to about 182 pounds for individuals with a large chest (40 inches). These values do not cover the whole range and are presented only to indicate the magnitude of the "lateral" factor of body, neglected in the ordinary height-weight tables.

For descriptive purposes the use of chest circumference is fully justified. For arriving at improved weight standards for evaluation of overdevelopment or underdevelopment of soft tissues, a purely bony measurement characterizing the lateral development of the skeleton should be used. Chest circumference includes not only the skeletal-frame factor, but also the soft tissues, principally fat. Thus, in the case of a man with a large chest circumference, due to massive fat deposits, the "standard" weight would be spuriously high and the subject's degree of overweight would be underestimated. For arriving at the degree of "obesity" or fatness, as differentiated from "overweight," the standard weight should take into account both the size of the skeleton and that of the muscle mass. The latter may be taken as being proportionate to the circumference of the limbs, corrected for the layer of subcutaneous fat (J. Matiegka, *Am. J. Phys. Anthropol.* **4**, *223 (1921)*).

Eighty-five hundred Army women, drawn from the Women's Army Corps and the Army Nurse Corps, averaging slightly below 27 years in age and 5 feet 4 inches in height, had a mean weight of about 131 pounds (F. E. Randall and E. H. Munroe, "Anthropometric Nomograph of Army Women,"

Report No. 148 (1949)). The nearest entry in the 1912 medico-actuarial tables indicates 129 pounds.

Of considerable interest is R. W. Newman's comparison of the medico-actuarial standards with white male inductees and two groups of separatees, measured in 1946 and 1949 ("The Assessment of Military Personnel by 1912 Height-Weight Standards," *Report No. 194 (1952)*). These groups are selected, not random or representative samples of the general population, with reference to age, and more particularly, with reference to the over-all fitness. The separatees, especially, should reflect the effect of physical conditioning combined with adequate nutrition and should approach the optimal physical state.

The inductees must have measured up to the Army induction medical standards. These standards are designed to prevent or at least minimize the acceptance of underweight personnel, while no rigid upper limit of body weight is imposed. The pertinent Army regulation on physical standards states that heavy men are acceptable "provided the overweight is not so excessive as to interfere with military training." The minimum acceptable weights correspond to from 86.0 to 90.5 per cent of the Army "standard" for height. For heights up to 6 feet the Army standards are close to or identical with the 1912 data for 20 year old men. For taller men the weight increments in the Army table are 4 pounds per inch while in the 1912 table the weight increment is 5 pounds per inch.

As could be expected, all three Army series examined by Newman were higher in weight than the 1912 medico-actuarial standards. Using relative weight as the criterion, obtained by expressing the actual weight or percentage of the 1912 standards for height and age, the mean for eight subgroups (ages 17 to 24) of white inductees was 102.8 per cent, with a range from 101.9 to 103.1 for different age groups. In view of the selection of the sample, which involved rejection of a certain percentage of the underweight registrants, Newman's data suggest that in the "general population" the 1912 tables largely retain their applicability, at least for young men.

Larger discrepancies were noted in the white and negro male separatees (mean relative weights of 104.4 and 105.0 per cent, respectively). "Overweight" was especially marked in the younger men and decreased with increasing age. This trend was more marked in the negro than in the white sample.

In the United States, a new set of height-weight standards, based on "recent" data compiled by the Equitable Life Assurance Society, was reported in *J. Am. Med. Assn.* **150,** *advertising section, p. 38, Sept. 13, 1952 issue.* A comparison with the 1912 standards indicates that the new average weights are higher for men, lower for women. As specific examples, the values for 25 year old men 5 feet 10 inches in height are 157 and 161 pounds, for women 5 feet 4 inches, 128 and 123 pounds, respectively. These are substantial differences and it is unfortunate that details of the study are not available.

On personal inquiry by the reviewer it was found that the sample included 53,008 men and 13,943 women between the ages of 10 and 65 who purchased ordinary insurance policies from the Equitable Life Assurance Society during the year 1940.

The current tables used by the Metropolitan Life Insurance Company are set up with five-year age groupings and are not directly comparable with medico-actuarial tables of 1912 which include increments of one year and one inch. However, approximative values can be obtained by using the midpoints of the age groups. For 25 year old men 5 feet 10 inches in height, the 1912 table gives 157 pounds, the Metropolitan table 156 pounds. For 50 year old men of the same height, the data are 171 and 171.5 pounds. For women 5 feet 4 inches at 25

years of age the values are 128 pounds and 127.5 pounds, at 50 years 144 and 143 pounds. In summary, there is no substantial difference. A scientifically documented report on the Equitable study is needed before valid conclusions may be drawn.

METABOLISM OF DIETARY CHOLESTEROL IN PATIENTS WITH HYPERCHOLESTEROLEMIA

In recent years attention has been drawn frequently to the significance of cholesterol in the causation of atherosclerosis (*Nutrition Reviews* **9,** *295* (*1951*); **10,** *148, 199* (*1952*)). A relationship has been suggested by the demonstration of relatively large quantities of cholesterol in atherosclerotic placques, the frequent association of increased concentration of the sterol in the serum of patients with atherosclerosis, and the production of lesions of atherosclerosis in experimental animals by cholesterol feeding and induction of hypercholesterolemia. It has been demonstrated that the concentration of certain lipoproteins in serum separated by ultracentrifuge technics is correlated positively with the occurrence of atherosclerosis. This relates to lipoprotein molecules with flotation rates in the ranges of S_f 12 to 20 and S_f 20 to 100 (J. W. Gofman *et al.*, *Science* **111,** *166* (*1950*); H. B. Jones *et al.*, *Am. J. Med.* **11,** *358* (*1951*)). These classes of lipoproteins contain cholesterol, but even larger proportions of the sterol are found in molecules of lower flotation rates, *i.e.*, S_f 0 to 12. Statistical analyses of such data by various groups have led to conflicting conclusions concerning the utility of these measurements in the diagnosis and management of atherosclerosis (*Nutrition Reviews* **9,** *167* (*1951*)).

An effort has been made to investigate pathways taken by ingested cholesterol in patients with both hypercholesterolemia and abnormal ultracentrifuge patterns of lipoproteins. These experiments have been recently described and are stated to demonstrate a quantitative defect in lipid metabolism in such patients (M. W. Biggs and D. Colman, *Circulation* **7,** *393* (*1953*)). The procedure utilized included administration of approximately 0.63 g. of tritium-labeled cholesterol dissolved in Wesson oil and emulsified in a standard quantity of milk and ice cream. Blood samples were taken at intervals thereafter for periods of three to four weeks and the tritium activity of the free and total cholesterol of serum was determined. During the course of the experiments the patients resided at home and consumed a usual diet. An ultracentrifugal analysis of the serum lipoproteins was done prior to feeding of the labeled cholesterol to each patient.

Ten individuals were studied. Three were classified as normal. (A question must arise in the mind of the reader concerning the designation of one subject as normal, because the serum cholesterol was recorded as 350 mg. per cent.) The remaining 7 patients suffered from disease processes which are often associated with disturbances in lipid metabolism. Included were 2 patients with atherosclerosis, 2 with xanthoma tendinosum, 2 with xanthoma tuberosum, and one with chronic glomerulonephritis. Each patient had hypercholesterolemia and/or an abnormality in the lipoprotein pattern.

A comparison of the specific activity of free cholesterol and of total cholesterol twenty-four hours after the administration of the labeled cholesterol demonstrated that in individuals with normal or nearly normal distribution of the lipoprotein fractions relatively less of the tritium-tagged cholesterol is present in serum as the esterified form than as the free sterol. In contrast, it is shown that the specific activity of the ester form equals or exceeds that of free cholesterol in other patients with abnormal

lipoprotein spectrums whose serum contains larger quantities of lipoprotein in the class of S_f 12 to 400. Responding in this fashion were the 2 patients with atherosclerosis, 2 with xanthoma tuberosum and one with chronic glomerulonephritis.

The authors found a significant correlation between the ratio of specific activity in free and total cholesterol and the ratio of concentrations of lipoproteins in classes S_f 0 to 12 and S_f 12 to 400. In contrast, the correlation coefficient failed to demonstrate a significant relationship between the ratio of cholesterol specific activity and the total cholesterol in serum. The conclusion is drawn that the mechanism accounting for the higher specific activity of serum cholesterol ester is related in some manner to the metabolic defect producing or associated with the abnormal lipoprotein pattern. Furthermore, it is stated that both manifestations of the defect in lipid metabolism may be totally independent of concentration of cholesterol in blood serum.

The rate of incorporation of the tritium tag in the cholesterol ester of the serum of patients with xanthoma tuberosum was similar to the enhanced rate found in patients with atherosclerosis. In contrast, the labelled cholesterol appeared in serum cholesterol of patients with xanthoma tendinosum at a rate comparable to the normal subjects. The authors propose, therefore, that on this evidence the two forms of xanthomatosis may result from fundamentally different metabolic defects.

DANGERS OF HYPODERMOCLYSIS

Parenteral nutrition ordinarily is taken to mean intravenous nutrition. However, intravenous feeding is not always feasible, either because usable veins cannot be found, or because in certain patients there is potential danger to the integrity of the circulation when fluid and electrolytes are injected rapidly into the vascular compartment.

For these reasons, hypodermoclysis (introduction of fluid into subcutaneous tissues), or, in common parlance, "clysis," is sometimes resorted to as a means of delivering such nutrients as glucose and amino acids to the patient parenterally (J. J. Weinstein, *Am. J. Surg.* **78,** *870 (1949)*). Perhaps the most common use of subcutaneous or intramuscular feeding is made by pediatricians in the supportive treatment of young infants. However, hypodermoclyses frequently are administered to chronically ill patients whose veins are no longer accessible. "Clyses" also may be given to patients in whom a precarious cardiac status precludes intravenous feeding.

Hyaluronidase has been shown to increase the rate of spread of fluid injected into subcutaneous tissues (F. Duran-Reynals, *J. Exp. Med.* **50,** *327 (1929)*; D. McClean, *J. Path. Bact.* **33,** *1045 (1930)*). By depolymerizing the mucopolysaccharide hyaluronic acid (a cementing agent in the intercellular ground substance), hyaluronidase ("spreading factor") facilitates the diffusion of solutions through the subcutaneous tissues. Accordingly, this enzyme has been widely used in conjunction with hypodermoclysis to enhance the disappearance rate of subcutaneously injected fluids (W. R. Webb, *Arch. Surg.* **65,** *770 (1952)*), and also to relieve patients of the discomfort due to local swelling at the site of injection (O. Hechter, S. K. Dopkeen, and M. H. Yudell, *J. Pediat.* **30,** *645 (1947)*).

Since it has been commonly assumed that hypodermoclysis is a benign procedure, many physicians have been startled to read of its potential dangers in a recent editorial (*J. Am. Med. Assn.* **151,** *644 (1953)*). In this editorial some of the work relating to the mechanism of absorption of subcutaneously injected fluids has been reviewed. It has been known for a long time that absorption

of isotonic glucose solution from an interstitial space is much slower than the absorption of isotonic sodium chloride solution (A. J. Schechter, M. K. Cary, A. L. Carpentieri, and D. C. Darrow, *Am. J. Dis. Child.* **46,** *1015 (1933)*). W. R. Webb, R. A. Lemmer, and R. Elman (*Surg. Gynec. Obstet.* **91,** *265 (1950)*) noted that subcutaneous injection of 5 per cent glucose solution was followed by a persistent increase in leg volume which lasted for several hours before absorption began, while injection of a similar volume of isotonic sodium chloride was followed by an immediate and progressive decrease in volume of the distended leg. Apparently, the delay in absorption of injected glucose in water occurs because glucose solutions so administered must come into equilibrium with body fluids before being further absorbed. This process entails withdrawal of water and electrolytes from the extracellular fluid space, including the vascular compartment.

Webb (*loc. cit.*) showed that the size of the leg after injection of a glucose solution was promptly reduced after local injection of hyaluronidase. Hyaluronidase causes more rapid diffusion of subcutaneously injected glucose; whether this is accompanied by significantly more rapid *absorption* has been the subject of speculation and study.

G. B. Forbes, R. W. Deisher, A. M. Perley, and A. F. Hartmann (*Science* **111,** *177 (1950)*) have shown that hyaluronidase increases the rate of entrance of subcutaneously injected Na^{24}-labeled sodium chloride solution into the circulation. On the other hand, W. E. Abbott and associates (*Surgery* **32,** *305 (1952)*) have studied the effect of subcutaneously injected glucose and invert sugar solutions on blood volume and serum electrolyte levels. They also observed the effect of hyaluronidase upon the absorption of glucose given by hypodermoclysis. Their findings are of special interest.

For example, one relatively healthy control subject was given 2000 ml. of a 5 per cent dextrose solution over a three and one-half hour period. The plasma volume six hours after the start of the hypodermoclysis was shown to be decreased by 21 per cent, as measured by the radioactive iodinated (I^{131}) albumin method. Hypotension, tachycardia and oliguria also developed in this subject and all these findings returned to normal only gradually. Roughly similar results were noted in other patients given 10 per cent sugar solutions subcutaneously and these changes seemed to be aggravated when hyaluronidase was given concurrently. It was concluded that hyaluronidase indeed causes subcutaneously injected glucose solution to diffuse over a greater area; however, the latent period during which equilibration with body fluids takes place still occurs, although somewhat more rapidly.

Thus, when an isotonic glucose solution is injected into an interstitial area the injection site becomes hypotonic with respect to such ions as sodium and chloride. These ions then rapidly diffuse into the hypodermoclysis region. However, since glucose traverses membranes at a considerably slower rate than that of the much smaller inorganic ions, water from the surrounding extracellular space, including the circulation, is drawn into the injection site. Hyaluronidase enhances this process by increasing the membrane area over which the exchanges take place, aside from any possible permeability effect.

Since solutions which contain only isotonic amounts of inorganic ions do not produce oligemia and its consequences when they are injected subcutaneously, they may be used safely for hypodermoclysis. Yet it now seems clear that when 5 and 10 per cent sugar solutions are given by hypodermoclysis a transient salt and water depletion of the vascular compartment occurs. Since oliguria, anuria and circulatory collapse may result from the subcutaneous, intramuscular, or intraperitoneal injection of sugar-in-water solutions, particularly in debilitated patients, administration of such

solutions by hypodermoclysis can no longer be regarded as a casual procedure.

Further study of the effects of subcutaneously injected solutions which contain *both* "isotonic" sodium chloride and larger molecules such as glucose, fructose, or amino acids obviously is needed. However, Abbott and his associates seem to have shown that the administration of sugar-in-water solutions by hypodermoclysis is a potentially dangerous matter. In the light of these findings it appears reasonable to give preference to the intravenous route whenever hypertonic solutions or solutions devoid of inorganic ions must be administered parenterally.

PSYCHOLOGIC FACTORS IN THE DEVELOPMENT AND TREATMENT OF OBESITY

In the field of "psychodietetics," the study of the complex relationship between psychologic processes and nutrition (M. F. Fritz, *Psychol. Clin.* **22,** *181 (1933)*), there are few, if any, problems which have more far-reaching practical implications than the motivational (psychodynamic) aspects of excessive food intake and of weight reduction.

Research on experimental animals has shown that lesions of the hypothalamus will result in marked increases in food intake (as much as three times the intake of normal rats), with overeating leading to obesity (A. W. Hetherington and S. W. Ranson, *Anat. Record* **78,** *149 (1940)*). Subsequent work not only confirmed the fact that the mechanism controlling food intake is located in the hypothalamus, but indicated that, within the hypothalamus, two mechanisms appear to be separable: (1) the nervous center in the *medial* portion, which may be concerned with "satiety," the destruction of which is followed by hyperphagia, and (2) a small region in the *lateral* hypothalamic area which may be regarded as the "eating center;" its destruction will completely abolish feeding behavior (B. K. Anand and J. R. Brobeck, 1951; reported by Brobeck, *Nutrition Symposium Series* **6,** *36 (1953), National Vitamin Foundation, New York*). Hyperphagia, induced by medial lesions, becomes aphagia, *i.e.*, the animals fail to eat, if both lateral hypothalamic areas are subsequently destroyed. The medial region appears to have the function of a regulator, capable of putting "brakes" (inhibition) on the eating behavior after sufficient amount of food has been taken.

If the observation is confirmed, it will represent a significant advance in our understanding of the neurophysiology of the regulation of food intake (E. F. Adolph, "Physiological Regulations," *Jacques Cattell Press, Lancaster, Penna. (1943)*). The hypothesis is proposed that the nervous centers of the lateral hypothalamus serve a facilitatory mechanism acting on feeding reflexes initiated by stimuli reaching the central nervous system from the sense organs of vision, olfaction, hearing, or touch. These reflexes are not completed unless the central facilitation mechanisms (via the lateral portions of the hypothalamus) are intact. In the absence of intact medially located neurons, which are assigned an inhibitory role, the reflexes continue in a facilitated state longer than they should. This failure of inhibition results in overeating, and, eventually, in hypothalamic obesity.

What are the changes, however, in animals deprived of food for some time or recently fed, to which the hypothalamic mechanism responds? At present, the nature of the changes which stimulate or inhibit the hypothalamus are obscure. It is apparent that the sensations from the stomach and duodenum are important. Intensive stimuli from the "empty" gastrointestinal tract are interpreted by the central nervous system

as "hunger pangs." However, the stomach has not an autonomous role in the regulation of food intake as is indicated by the fact that removal of the stomach does not abolish feeding behavior. M. I. Grossman, G. M. Cummins, and A. C. Ivy (*Am. J. Physiol.* **149,** *100 (1947)*) have demonstrated that a complete denervation of the upper gastrointestinal tract does not greatly impair the dog's ability to regulate food intake.

E. F. Adolph (*Am. J. Physiol.* **151,** *110 (1947)*) has shown that normal animals have a remarkable ability to adjust their food intake to the composition of the diet. This regulation is not perfect, and while the caloric value of the ingested food is important, the mechanism for signaling the intake of a certain amount of potential energy is not known. The possible role of two other factors has been considered: the rise in heat production related to the "specific dynamic action" of food, and the amount of carbohydrate available to the central nervous system (the glucostatic hypothesis; *see Nutrition Reviews* **11,** *55 (1953)*). At present it is not clear whether some one factor will explain all the observations, or whether a multiple-factor hypothesis will be needed.

H. H. Mitchell (*J. Clin. Nutrition* **1,** *66 (1952)*) feels that attempts to explain on physiologic grounds the inception of obesity have so far been unsuccessful. At any rate obesity, uncomplicated by endocrine dystrophy, has not been shown definitely to be associated with a lower basal metabolism, a lowered specific dynamic action of food, or a greater efficiency in the digestion of food. While in very rare instances "organic" factors, such as brain tumors, will produce morbid hunger (bulimia), and pancreatic tumors, causing hyperinsulinism, will lead to hyperphagia and eventually result in obesity, the preponderance of evidence is in favor of a motivational (psychodynamic) etiology of excessive food intake (H. W. Brosin, *Nutrition Symposium Series* **6,** *52 (1953), National Vitamin Foundation, New York*). It is not unreasonable to regard gross overeating ("phagomania") as akin—etiologically, clinically, and therapeutically—to chronic alcohol addiction.

A review of the literature on obesity, stressing the psychosomatic approach, was presented by A. P. Burdon and L. Paul (*Psychiatric Quarterly* **25,** *568, Utica, N. Y. (1951)*). An important fact is that food constitutes means for direct ("real") and symbolic satisfactions. To a young child food and love are synonymous, and the offering and receiving of food acquires powerful emotional significance. In the adult, anxiety readily disturbs the normal eating pattern. In individuals whose personality is so patterned that oral gratification through eating allays anxiety, prolonged emotional stress is likely to lead to obesity. In the predisposed persons a variety of situational factors (fear of pregnancy, death of a loved one, hospital experience, and financial, social, or intellectual failures) may precipitate overeating.

W. W. Hamburger (*Med. Clin. No. America* **35,** *483 (1951)*) classified overeating into four types of responses: (1) a response to a nonspecific emotional tension, (2) a substitute gratification to intolerable life situations, (3) a symptom of underlying emotional illness, and (4) an addiction to food. It should be noted that moderate obesity becomes common as people age and decrease their activity, without reducing their food intake (*Nutrition Reviews* **11,** *100 (1953)*). This should be differentiated from overweight which is produced by compulsive eating and which is an expression of emotional maladjustment.

Excessive weight brings to the patient many griefs, physical and personal. It makes him more susceptible to disease. He is faced by medical disapproval and social condemnation. Yet it is well known that the treatment of obesity is frequently ineffective. The neglect of the psychologic component appears to be the principal reason for the frequent failure to reduce, and, equally importantly, to maintain the

reduced weight level. Burdon (*loc. cit.*) believes that the unpleasantness of a reducing regimen is rarely due to true hunger, but to the fear of emotional deprivation and the frustration of the patient's oral wishes and desires. While treatment of every obese individual must be psychiatrically oriented to be successful, in some cases the skills of the internist or the family physician will fully suffice. However, many individuals need considerably more extensive professional psychiatric treatment. In Burdon's terms, therapy should allow the patient to find more dynamic outlets for his erotic and creative drives than are represented by the static form of physical largeness.

The psychologic aspects of reducing were considered by H. Bruch (*Psychosomatic Med.* **14**, *337 (1952)*), whose research publications on obesity, especially in children, cover some fifteen years. She commented that reducing, the deliberate effort to lower body weight, has become an all-American preoccupation and a profitable business to numerous slenderizing institutions, not always weighted down by high ethical standards. While patients under competent medical supervision may experience serious difficulties in attempts to reduce, commercial enterprises may induce the victims trapped in their own fat into senseless or even dangerous reducing procedures.

Bruch (*loc. cit.*) starts with the important point that the success or failure to reduce depends, among other factors, on the meaning of weight reduction to the patient. Overweight individuals who consider weight reduction do not represent psychologically a homogeneous group. An emotionally adequately adjusted person, usually only moderately overweight, approaches the goal of losing weight in a rational manner. He wants to reduce for realistic reasons, commonly out of considerations of health or appearance. Even then the fulfilment of the goal is not easy and simple, and many patients need psychologic support and supervision. Bruch cites Clemence Dane's bon mot "Being witty is like being slim—it's beastly hard work."

The problems of weight reduction are much more severe for individuals who are grossly obese. Psychoanalysis of these patients frequently reveals a grave distortion of their sense of reality. There is a characteristic gulf between their "level of aspiration" and their ability to live up to their ambitions, and overeating becomes the most important means for relieving the dissatisfaction. The result of the repeated cycles of frustration and overeating is increased obesity. The individual suffers from deep seated disgust and feels guilty for being weak and gluttonous. The condition of becoming thin may become associated with dreams of positive accomplishment and success. Weight reduction is then not regarded as a simple physiologic fact of losing weight but becomes the magic key opening the doors to the fulfilment of exaggerated hopes and daydreams; it acquires an irrational meaning.

As a rule diet is broken before any substantial amount of weight loss is achieved. But, unless a corrective appraisal of the aspiration level has been achieved, many of the persons who do get their weight down continue to be dissatisfied and frequently resume overeating in the spirit of despair. While some of Bruch's psychoanalytic interpretations and facile verbal associations may be challenged, such as the implied identification between the yearning for "greatness" and the achievement of the "bigness" of obesity, her clinical experience cannot be disregarded. If the weight reduction is rigidly enforced in a high-pressure atmosphere of a beauty salon, serious mental illness may be precipitated. Her remarks about the grave psychologic dangers of commercial exploitation of the weight problem are based on a number of observations on young girls who became seriously emotionally disturbed while trapped in the process of mechanical, psychologically unsound reducing. These case studies are

presented in detail and illustrate the conflicts and psychiatric involvement which may be associated with weight reduction. In some individuals a persisting obesity, undesirable from the strictly medical (somatic) point of view, may serve as a wall against a serious mental disturbance and may be preferable as a lesser evil.

INTESTINAL MOTILITY AND ABSORPTION

Normal gastrointestinal function is of prime importance in the maintenance of good nutrition. The mechanisms which lead to absorptive failure in certain disease states are poorly understood. The role of increased or decreased motility of the small intestine in producing malabsorption has not been delineated despite the fact that hypermotility in ulcerative colitis and hypomotility in nontropical sprue have been frequently described. In this regard two attempts to determine the effect of motility upon absorption have recently been reported.

In an attempt to determine the importance of hypermotility in patients with ulcerative colitis, K. Uyeyama, J. E. Giansiracusa, T. L. Althausen, and H. A. Harper (*Gastroenterology* **23**, *143 (1953)*) have carried out vitamin A, galactose and methionine tolerance studies in patients with this disease and compared the results with those in 13 patients with hypermotility of the small bowel as determined by the passage of barium to the cecum in one hour or less, or to the splenic flexure of the colon in six hours or less. Although the fasting vitamin A blood levels in most of the colitis patients were normal, averaging 113 I.U. (range 32 to 90) per 100 ml., the tolerance curve was abnormally flat in 23 of 31 subjects tested. The average maximum rise for those with flat curves was 99 I.U. (range 0 to 200), while in the 8 patients with normal curves the average maximum rise was 447 (260 to 630) I.U. per 100 ml. There was no relationship observed between the vitamin A tolerance and the severity of the disease.

The effect of administering Tween 80 with the vitamin A was determined in 19 patients. Of these, 13 had abnormal curves before Tween 80 administration, while 6 were in the normal curve group. Five of the former and 2 of the latter group showed a significant change in the blood levels obtained with the use of the detergent, significance being defined as an increase of 100 I.U. of vitamin A per 100 ml. of blood at four hours. The average net effect of Tween 80 administration upon the maximum rise was slightly greater in the normal curve group of patients (147 I.U.) than in the flat curve group (85 I.U.), but there was considerable overlap in the responses of patients in the two groups.

The effects of ACTH and cortisone, which have been reported to produce temporary remissions in ulcerative colitis, were also studied in relation to vitamin A tolerance. In 6 patients so treated 4 were found to have completely normal curves as a result of therapy while one patient demonstrated improvement in his curve to a level less than normal. Despite a good clinical remission in the sixth patient, the ACTH produced no improvement in vitamin A tolerance. Another patient treated with ACTH and cortisone derived no clinical benefit or change in his tolerance curve, but did have a good response in both respects to compound F. No relation of improvement in vitamin A tolerance curve to cessation of diarrhea could be demonstrated in that in some patients relief of the diarrhea preceded the tolerance improvement while in others the reverse was true.

In 24 of 28 colitis patients studied, the galactose tolerance curve was abnormally high while methionine tolerance was found to be lower than normal in 6 of 11 patients.

No data were given by the authors as to whether these findings correlated with the vitamin A findings and no studies of these latter substances during hormone therapy were reported.

Somewhat in contrast to the above findings were the results obtained in patients with intestinal hypermotility. In this syndrome 11 of 13 patients revealed normal vitamin A curves while the remaining 2 had figures slightly below normal. Of interest is the fact that one patient with an ileosigmoidostomy with a transit time of twenty minutes for barium to travel from mouth to rectum demonstrated a normal curve. Oral galactose tolerance curves increased in 11 of the 14 so tested while methionine produced normal curves in all 6 patients studied.

These studies indicate that fat absorption as it is measured by vitamin A tolerance may be adversely affected in colitis, but amino acid absorption is less often affected while carbohydrate absorption may be enhanced. The authors state that normal intravenous galactose tolerance tests in many of their colitis patients rule out the possibility that the increased blood levels of galactose were a result of poor liver function. While carbohydrate absorption enhancement may be due to intestinal hypermotility, as reported in both colitis and hypermotility patients, it would appear that fat absorption is relatively little affected by this aspect of gastrointestinal function. The divergence of findings in the two groups of patients studied suggests instead that malabsorption in colitis is due to a defect other than increased motility of the small bowel.

Another approach to the problem of absorption and motility relationships is reported by A. J. Cummins and T. P. Almy (*Gastroenterology* **23,** *179 (1953)*). In their investigation normal subjects and sprue patients were studied by the "isolation" of a 45 cm. length of bowel by the use of intubation and balloon obstruction at the distal end of the segment. The material tested is injected proximally and aspirated at the end of thirty minutes by suction through a lumen at the distal or balloon end. By use of this technic glucose absorbed from the test dose of glucose and methionine is measured by subtraction of the amount recovered from the injected dose. Hypermotility in the intestine was produced by physostigmine or urecholine in 3 normal subjects and in 2 patients with sprue with the result that all revealed increased glucose absorption over previous control studies and 4 of 5 showed increased methionine absorption, one normal subject failing to increase his absorption of the latter substance.

Although they were unable to eliminate completely the possibility of so-called "telescoping" of the intestine about the balloon with resultant increase in the area of bowel surface thus presented to the substance to be absorbed, the authors state that observations in dogs similarly intubated and treated revealed no such effect. Thus they suggested that increased absorption was probably related to increased motion of the intestine.

In attempts to study the effect of hypomotility produced by banthine in this same manner the authors found that no material could be aspirated from the distal end of the segment of intestine, probably because passage was so delayed. They therefore undertook glucose tolerance studies in normal subjects by introducing glucose through a tube into the duodenum after hypomotility was produced by banthine administered intravenously. No alteration in the glucose tolerance curve was observed in such studies nor in an intravenous glucose tolerance study carried out under the effect of this anticholinergic drug.

The authors conclude that the induced hypermotility may be responsible for the increased absorption of glucose and methionine in both normal persons and in patients with sprue. They are careful to point out, however, that these findings may have no relation to the absorption of fat. Further, they emphasize that the induction of hypomotility does not produce lowered glucose

tolerance curves in normal individuals and, therefore, the hypomotility observed in sprue patients is probably not the cause of flat glucose tolerance curves in this disease. Hypermotility produced by the use of drugs in these patients was found to increase glucose absorption as measured by the disappearance of this substance from the intestinal material aspirated, but the induction of hypermotility by cholinergic drugs probably does not correct the basic defect in sprue.

These sets of investigations which attack the motility problem from different approaches confirm each other in some but not all respects. Carbohydrate absorption was found in both studies to be increased by the presence of hypermotility. Methionine absorption appeared to be decreased or normal in the hypermotility of ulcerative colitis or in patients with spontaneous hypermotility, while it was increased in drug-induced hypermotility. It is perhaps unfortunate that fat absorption has not yet been studied by the technic of Cummins and Almy, but from the data presented by the other group of workers it would appear that motility is not a very great factor in the gastrointestinal management of this substance. Extension of these investigations to include other types of malabsorption would appear to be desirable, however.

NUTRITION AND INDUSTRIAL PERFORMANCE

Recent literature on the influence of nutrition on work performance has been reviewed by E. Simonson (*Nutrition Symposium Series* **3,** *72 (1951), National Vitamin Foundation, New York*). From the point of view of industrial medicine the problems of nutrition were considered by N. Pardon (*Médecin d'usine* **14,** *249 (1952)*). For a systematic presentation of the relationship between the nutrition of industrial workers and their health and efficiency the reader will find a reliable guide in Magnus Pyke's "Industrial Nutrition" (*Macdonald and Evans, London (1950)*). It reviews the available evidence as to how different types of work affect nutritional requirements and how meals can best be arranged within the varied conditions of industrial life to provide different groups of people with adequate food.

The problem of meal habits and the effects on performance were discussed in detail by R. C. Hutchinson (*Nutrition Abstr. Rev.* **22,** *283 (1952)*), citing some one hundred references. Some of Hutchinson's material has been covered in *Nutrition Reviews* **8,** *167 (1950)*. The new material he provides consists of a graph representing a "typical" daily production curve, indicating the rise in the level of output during both the morning and afternoon work period, followed by a "fatigue" decrement in the latter part of each period. Of particular interest is the graphic representation of the changes in the output level during the afternoon work period. A full midday meal is reflected in the production curve by a "flattening," while after a light snack the production curve rises more steeply. No details are given as to the type of operation on which the observations were made, the number of workers, the actual sizes of the meals, etc. The graph has been prepared by the author from descriptive information received from unidentified sources. The difference in working rates under the two conditions are shown, but the data on which the graph is based are such that a quantitative comparison could not be made. Thus, scientifically, we have not progressed beyond the common-sense observation that ingestion of a large meal is frequently associated with a feeling of drowsiness, lack of coordination, and disinclination for mental or physical effort.

While verified quantitative observations are scanty or lacking, the "explanations" of

the phenomena associated with ingestion of a heavy meal are at hand—be it a temporary "brain anemia" due to the demands for blood by the digestive organs, or postprandial hypoglycemia ascribed to the oversecretion of insulin stimulated by the sharp rise in blood sugar following the consumption of a heavy meal. The available evidence is primarily indirect and inconclusive. It may be noted that J. F. Pearcy and T. D. Allen (*Am. J. Physiol.* **82,** *56* (*1927*)) found that distension of the stomach with air alone produces effects similar to those following the ingestion of a large meal—drowsiness, relaxation, warm skin, diminished knee jerk, decreased attention and lowered visual efficiency.

Even in the absence of detailed scientific evidence it will be readily agreed that very long periods between eating, for example, a two-meals-a-day schedule, are undesirable for a large majority of individuals and are likely to be associated with feelings of emptiness and weakness, restlessness, irritability and decreased ability to concentrate. Also, large meals appear undesirable, especially in occupations in which the work is done in a bent or crouched posture. In heavy industrial and agricultural work preventing the blood sugar level from falling may be more important than in light or moderately heavy work. In these occupations a consumption of five to six meals a day is a common practice (*cf.* Pyke, *loc. cit.*). A regimen of six meals daily is recommended by Hutchinson.

Evidence is available for the superiority of smaller meals spaced at shorter intervals in withstanding intense cold (H. H. Mitchell *et al.*, *Am. J. Physiol.* **146,** *84* (*1946*)), in hot environments (B. Schlegel and H. Böttner, *Ernährung* **6,** *177* (*1941*)), and at high altitude (H. R. Bierman, *War Med.* **3,** *1* (*1943*); *cf.* Mitchell and M. Edman, "Nutrition and Climatic Stress," *C. C. Thomas, Springfield, Ill.* (*1951*)). A. Grollman (*Am. J. Physiol.* **89,** *366* (*1929*)) observed that a large meal caused an immediate and large rise in cardiac output and it appears that the extra strain, imposed on a diseased heart, could account for the attacks of angina pectoris which sometimes occur at such a time. In studies not cited by Hutchinson, E. Simonson and A. Keys (*Circulation* **1,** *1000* (*1950*)) have shown in normal men that even the ingestion of a meal of moderate size has definite effects on the electrocardiogram (increased heart rate, increased R amplitudes, decreased T waves, and a right QRS axis shift). Simonson and C. A. McKinlay (*Ibid.* **1,** *1006* (*1950*)) examined the possible utility of a meal test as a mild and convenient form of "stress" in clinical work. The hypothesis was confirmed, with a high incidence of abnormal electrocardiographic changes after a meal in a large number (75 per cent) of patients with suspected or proved heart disease (coronary insufficiency, arterial hypertension, abnormal electrocardiogram at rest). There was a tendency toward appearance of multiple abnormal changes with increasing myocardial involvement. The response of the T wave was most frequently aberrant.

The relationship between nutrition and production capacity was considered also by A. Jung (*Gesundheit* (*Zürich*) **31,** *12* (*1951*)), although the presentation was centered on the analysis of food consumption in Switzerland. A normal young man engaged in light work (daily caloric expenditure of 2400 calories) was considered as the caloric reference point ("nutritional unit") and the caloric needs of men engaged in moderate, heavy and very heavy work were expressed in terms of multiples of this unit (with coefficients of 1.25, 1.5 and 2.0, respectively). For a married couple without children the caloric needs were expressed as 1.80 units, and so forth.

The practice of between-meal feeding is regarded as a positive factor in production efficiency, the mid-morning snack being especially important when the work starts very early in the morning. However, in some cases the interruption of work requires a new "warm-up," with a slowly rising pro-

duction level, so that the practice is of questionable merit, as far as total work output is concerned. Unfortunately, this important point was not documented by actual data. It is pointed out that in intellectual work the mood plays an important role and that it can be affected, to a certain degree, by food intake. While the effects of tea and coffee are well known, Jung believes that meat has, in many people, a stimulating effect. While the practical importance of these factors is undeniable, scientific investigations are woefully meager in comparison with the wealth of medico-culinary lore. In this instance, Jung's statement on the effects of meat intake is supported, at least in part, by the observations of G. W. Thorn, J. T. Quinby, and M. Clinton (*Ann. Int. Med.* **18,** *913* (*1943*)) that, in contrast to a high carbohydrate and—to a lesser extent—a high fat breakfast, an isocaloric high protein meal was not followed by symptoms of hypoglycemia; there was a high metabolic rate and an improved sense of well-being. However, by a proper selection of the material, documentation could be provided for the supremacy of other nutrients. Thus C. G. King *et al.* (*J. Aviation Med.* **16,** *69* (*1945*)), in their studies of altitude tolerance by airmen, concluded that performance scores were best after high carbohydrate meals. The statement that for the performance of muscular work a diet rich in carbohydrate is preferable to a high fat diet is perhaps one of the few fairly safe generalizations in this segment of nutritional research, but even here exceptions have been found.

MUSCLE DYSTROPHY IN CALVES

British workers have recently conducted a series of studies to reinvestigate the importance of vitamin E in the nutrition of the calf. In initial studies they showed that the severity of muscular dystrophy in calves was increased by the addition of small quantities of cod liver oil to the ration (K. L. Blaxter, P. S. Watts, and W. A. Wood, *Brit. J. Nutrition* **6,** *125* (*1952*)). The muscular dystrophy observed by Blaxter and associates is closely comparable to a naturally occurring disease in calves known as *weisses Fleisch* (white flesh) and has been reported from several European countries.

Recent studies were conducted to establish whether, under practical conditions, muscular dystrophy would follow when cod liver oil was added to the diet of calves. This practice had been recommended in recent years by the British Ministry of Agriculture and Fisheries as a means of insuring an adequate intake of vitamin A for calves fed skim milk rations in early life (K. L. Blaxter, W. A. Wood, and A. M. MacDonald, *Brit. J. Nutrition* **7,** *34* (*1953*)).

Twenty-eight Ayrshire bull calves were allocated by lot to the five following treatments as a daily ration: Lot 1, 750 g. of dried skim milk powder (dissolved in tap water) and arachis (peanut) oil solution containing 2000 I.U. of vitamin A and 200 I.U. of vitamin D; lot 2, same as lot 1, plus 50 mg. of alpha-tocopherol acetate; lot 3, 750 g. of dried skim milk powder, plus 1 ounce of cod liver oil daily; lots 4 and 5 were the same as lot 3, except that the cod liver oil was increased to 2 and 4 ounces, respectively. The allowance of skim milk was increased to 1000 g. after fifty days.

The calves were placed on the experimental rations when they were 12 to 15 days old, having previously received a ration of whole milk. At 2 to 3 days of age, each calf had also received a supplement of 4 capsules of halibut liver oil, each containing 4500 I.U. of vitamin A and 450 I.U. of vitamin D.

Seventeen of the 18 calves in lots 3, 4 and 5 (cod liver oil-supplemented) developed symptoms of muscular dystrophy. The physical signs observed in the most severely

affected animals were abnormal gait, flexion of the knees, winged scapulas, outward rotation of the hocks, and inability to rise. Symptoms that had not been observed in the earlier studies were acute abdominal respiration, severe heart attacks with consequent prostration, and presumed dystrophy of the lumbar muscles.

The average number of days of cod liver oil feeding for the production of symptoms of muscular dystrophy ranged from twenty days, when the daily intake of oil was 4 ounces, to thirty-six days when the oil intake was 1 ounce. The feeding of cod liver oil had no adverse effects on body weight gain. The calves affected with muscular dystrophy did, however, exhibit edema of the musculature.

The appearance of the affected muscles was similar to that described in the earlier publication (Blaxter, Watts and Wood, *loc. cit.*). The dystrophic lesions in the muscle were highly variable. In some muscles they were circumscribed and in others the lesions ran parallel with the muscle fibers. In severe cases only the outer shell remained normal, whereas in others it was this area alone that was dystrophic. Attempts to classify muscles and muscle groups according to the degree in which they were affected failed to reveal any consistent pattern. There was, however, a certain degree of bilateral symmetry in the pattern of dystrophic lesions. Besides the severe dystrophy of the limb muscles, dystrophy of diaphragmatic and intercostal muscles was also observed. This was believed to be an explanation for the acute abdominal respiration.

The heart failure of some of the calves is of interest, since a comparable sudden heart condition has been reported by others in cattle subjected to vitamin E (tocopherol) deficiency (*Nutrition Reviews* **9**, *76* (*1951*)). A grey heart muscle, sometimes with a patchy appearance, which on microscopic examination revealed grainy vacuolar degeneration was noted in the dystrophic calves that exhibited signs of heart failure.

None of the calves in lots 1 and 2 receiving peanut oil with or without tocopherol exhibited any clinical signs of muscular dystrophy, although a few minor dystrophic lesions were noted in some of these animals upon post mortem examination. The average muscle concentration of creatine in the three lots of calves receiving cod liver oil ranged between 327 and 355 mg. per 100 g., compared with values of 380 and 365 mg. per 100 g. in the muscles of the calves receiving the peanut oil with or without tocopherol.

Considerable variation was noted in the time of onset and the physical signs of muscular dystrophy. Since the animals were procured from various sources, no data were available as to whether or not the calves might have varied in their vitamin E reserves. An additional study was conducted (K. L. Blaxter, F. Brown, and A. M. MacDonald, *Brit. J. Nutrition* **7**, *105* (*1953*)) to determine whether the normal nutritional practices of farms in Scotland could result in any appreciable variation in tocopherol reserves of calves.

Three farms with widely differing feeding and management systems cooperated in the study. The results of analyses of the blood serum of the cows and calves, together with analyses of colostrum and milk of the cows, made it clear that very large differences in the tocopherol content existed among the animals on the three farms. A ten-fold variation in serum tocopherols was noted in 4 day old calves. The average serum tocopherol content in the calves on farm 3 was 60 micrograms per cent contrasted with a value of 270 micrograms per cent in calves on farm 1 and 180 micrograms per cent in calves on farm 2. These were values obtained in late winter. Values in late summer or autumn were nearly alike on the three farms, averaging about 230 micrograms per 100 ml. of blood. Farms 1 and 3 differed in herd feeding management in that the former resorted to winter grazing, whereas farm 3 maintained its herd on hay, straw, mangolds, and concentrates during the winter.

It was further noted that the feeding of

mangolds to cows in lactation depressed the tocopherol content of the milk fat. Generally, the tocopherol content of milk from cows in full lactation showed a marked rise when the cows were permitted to graze after dry ration feeding. In general, there was good correlation between the tocopherol content of the blood serum of 4 day old calves and the tocopherol content of the serum of their dams.

Although no evidence of muscular dystrophy was apparent in any of the calves on the farms under study, there was a lowering of the muscle and heart creatine content in calves born in late winter when the tocopherol reserves were generally most depleted.

Although this most recent study was extensive and attempted to take care of all possible variables under practical farm conditions, the results did not establish any clear-cut correlation between the tocopherol concentration of serum and milk of dams, the tocopherol serum levels of calves, and the appearance of early lesions of muscular dystrophy. The only interpretation at present permissible is that some environmental factor influences vitamin E metabolism and is thus involved in the etiology of muscle dystrophy in calves. One of the environmental factors considered by the authors was environmental temperature. Irrespective of the blood levels of the dams, calves appeared to be more susceptible to muscular dystrophy in late winter when they were born in cold weather than when they were born under more favorable weather conditions.

SYNTHESIS OF VITAMIN B_{12} BY TUMOR TISSUE

The nutritional approach to an understanding of the etiology of cancer still seems promising. If a qualitative or even a quantitative difference between the metabolism of normal and that of neoplastic tissue could be demonstrated, a basis for more precise study of the disease would be at hand. Through the use of antimetabolites it is possible to select analogs of an indispensable substance concerned in cellular metabolism, thereby interfering with the proliferation of the involved tissue cells. In a recent report (D. W. Woolley, *Proc. Nat. Acad. Sci.* **39,** *6 (1953)*) this line of thought has been developed with reference to the divergence in metabolism between normal and neoplastic tissue.

In order to avoid the possibility of a factor of similar constitution acting vicariously for the one under direct consideration, a precursor for the synthesis of more than one vitamin should be selected. The substance used as an experimental model in the study here described was 1,2-dimethyl 4,5 diaminobenzene, the common precursor of riboflavin and vitamin B_{12}. It has been shown (D. W. Woolley and A. Pringle, *J. Biol. Chem.* **194,** *729 (1952)*) that analogs such as dichlorodiaminobenzene and dimethyl-4-amino-5 hydroxybenzene restrict the growth of organisms capable of elaborating their own riboflavin and vitamin B_{12}, but leave unaffected those species requiring the preformed vitamins. If, then, the neoplasm in question could be shown to possess the ability to synthesize riboflavin and vitamin B_{12}, it would be susceptible to the peculiar action of the antimetabolite whereas this would not be true for the host animal.

Since the nutritional requirements *in vitro* could not be determined, advantage was taken of the fact that vitamin B_{12} is transmitted to the offspring by female mice in sufficient quantity to satisfy the needs of the young even when the latter are restricted to a vitamin B_{12}-deficient diet. This does not occur if the mother is given the deficient ration from the day of mating, and under such circumstances the young soon

show signs of avitaminosis. Thus, if a tumor-bearing pregnant female is maintained on a vitamin B_{12}-deficient diet and the young show no signs of the deficiency, the conclusion can be drawn that the neoplasm is synthesizing the vitamin in sufficient amount to satisfy the needs of the young. There is then at hand an experimental device for influencing the growth of the tumor through nutritional means by way of the appropriate antimetabolite.

It was demonstrated that the young of normal Swiss mice mothers on the vitamin B_{12}-deficient ration (L. M. Zucker and T. F. Zucker, *Arch. Biochem.* **16,** *115 (1948)*), themselves maintained on this diet, showed a lower rate of growth per week than did similarly fed progeny of normal mothers receiving the vitamin throughout gestation. In like manner, female Swiss mice bearing spontaneous mammary tumors of proven malignancy were bred and maintained on the ration deficient in vitamin B_{12} until the young were weaned and the progeny again were restricted to the deficient ration. It is evident, on the basis of data from large numbers of mice, that the young from the tumor-bearing animals grew more rapidly than did those from normal mothers, strongly suggesting that vitamin B_{12} was supplied to the host and transmitted to the young. The same phenomenon of enhanced growth of young from tumor-bearing females was observed with C_3H and C-strain mice with spontaneous mammary tumors.

This type of experiment was carried out with female Swiss mice in which the same tumor had been transplanted on the day of mating. However, there was no difference between the subsequent rate of growth of the young from tumor-bearing mothers and that of the young from normal females. The same failure to demonstrate a difference was reported when the young of females with erethane-induced lung adenomas were compared with the progeny of normal mothers under the experimental conditions described. However, the success with the spontaneous mammary tumors suggests a promising chemotherapeutic device for influencing the growth of neoplasms, which is worthy of further exploration.

EFFECT OF AN EXCESS OF VITAMIN A ON CHICK ECTODERM GROWN IN TISSUE CULTURE

It has been demonstrated that the addition of an excessive amount of vitamin A to a tissue culture medium in which rudiments of bone from chick embryos are grown produces changes in the cartilaginous matrix qualitatively similar to those in bones of animals made hypervitaminotic by feeding large quantities of vitamin A (H. B. Fell and E. Mellanby, *J. Physiol.* **116,** *320 (1952)*; *Nutrition Reviews* **10,** *343 (1952)*). The use of the tissue culture technic is associated with distinct advantages in the study of the influence of a vitamin on tissues. It permits direct observation of the tissue, facilitates manipulation of conditions, and makes for ready reproducibility.

After the successful demonstration of this effect of an excess of vitamin A on bone in tissue culture it was to be anticipated that similar studies would be made of other tissues known to be influenced by a deficiency or an excess of the vitamin. A recent report from the same laboratories concerns the effect of high concentrations of vitamin A on chick ectoderm (H. B. Fell and E. Mellanby, *J. Physiol.* **119,** *470 (1953)*).

Deficiency of the vitamin has long been known to produce epithelial changes with occurrence of keratomalacia, xerophthalmia and a generalized replacement of the normal epithelium by keratinizing stratified squamous epithelium. Experimental observations have indicated that increased concentrations of the vitamin given by mouth or applied

locally to the skin may inhibit keratinization of the epidermis (A. Studer and J. R. Frey, *Schweiz. med. Wchnschr.* **79,** *382 (1949)*; J. D. Sabella, H. A. Bern, and R. H. Kahn, *Proc. Soc. Exp. Biol. Med.* **76,** *499 (1951)*).

Explants of ectoderm were taken from the trunk and limbs of 6 or 7 day old chick embryos. These were grown on a medium composed of three parts of chicken plasma and one part of a concentrated extract of chick embryo prepared in Tyrode solution. Vitamin A was added to the plasma either as the alcohol or acetate dissolved in ethanol. An equivalent quantity of ethanol was added to the plasma used in control studies. Final concentration of the vitamin in the plasma was from 200 to 350 I.U. per 100 ml. in control experiments (the normal range of concentration of the vitamin in chicken plasma) and from 1000 to 3000 I.U. per 100 ml. in the hypervitaminotic plasma. The cultures of ectoderm were transferred to new media either three or four times a week. In all experiments when ectoderm was grown in the presence of high concentrations of vitamin A, a similar specimen was taken from the opposite side of the same embryo and grown in a control medium without added vitamin A.

The first series of experiments sought evidence of an effect of excess vitamin A on differentiation of the chick ectoderm. Control explants grown on watch glasses continued to develop and differentiation of the epithelium occurred at a more rapid rate than in the epidermis of the intact embryo. The growing explant spread over the surface of the culture medium and increased in thickness. By the seventh day in culture the epithelium was folded and covered in part by an opaque layer of cornified material. Dome-shaped feather papillae appeared on the fourth day and proceeded to elongate, forming a bottle-shaped appendage attached to the skin, but they did not go on to the more complete differentiation seen in the intact embryo *in ovo*. The connective tissue formed two layers, with an outer zone of reticular tissue corresponding to the dermis and an inner compact mass of subdermal connective tissue. The connective tissue degenerated if the explant became completely epithelialized.

Ectoderm grown on a watch glass in a medium containing the excess of vitamin A grew much as did the control explants for the first four days. However, the epidermis failed to keratinize and swelling or edema of the subepidermal tissue occurred. The feather papillae became enlarged and distorted in appearance. After eleven to fourteen days in this medium there was still no gross evidence of cornification. The edema decreased and the connective tissue was more compact. A thick clear secretion appeared about the tissue. Histologic examination confirmed the absence of keratinization. By the seventh day of culture, cells of the epithelium assumed cuboidal configuration and contained secretory materials. This change was more pronounced by the ninth day and the epithelium no longer resembled the cornified squamous layer of the control explants. Cells were cuboidal or columnar in type and the structure became that of a secretory membrane. Sheets of cells became detached from the basal layer. Certain cells were seen to be ciliated and many contained secretory vacuoles.

When explants were grown by a second technic—hanging drop preparation—similar effects of vitamin A present in excess in the medium were noted. However, the tissue grew to form vesicles, which were distended with mucoid secretion and lined with secretory epithelium composed of columnar cells resting on a flattened layer of cells below which was a basal layer of cuboidal cells.

The authors compared the changes produced by high concentrations of vitamin A on ectoderm in tissue culture with another developing secretory membrane, that of the nasopharynx. The embryologic differentiation of nasal epithelium in the normal fowl includes a sequence of events comparable to

that observed in the ectoderm grown in an excess of vitamin A. The epithelium first contains two layers of cells. Additional layers of cells then develop, surface layers of flattened cells are shed and replaced by a cuboidal or columnar epithelium which is ciliated and assumes secretory capacities. The conclusion seemed warranted that the hypervitaminotic growth medium caused differentiation of ectoderm from the trunk and limbs into a mucous secreting membrane histologically similar to that of the nasal cavity.

It was possible to reverse the induced differentiation into secretory epithelium by transplanting the tissues to a growth medium to which no supplements of vitamin A were added. Cultures grown for seven to eleven days in an excess of the vitamin were transferred to the control medium. For the first several days after transfer the development of the ciliated secretory epithelium continued. However, there followed failure to produce new cells of this type. The basal cells of epithelium multiplied to form squamous epithelium lifting the secretory layer and causing its degeneration. The sequence of events was that of reversion to the epithelial structure of the ectoderm grown in a medium to which no vitamin A was added.

The significance of these ingenious experiments lies in their assignment to vitamin A of a major role in determining the type and direction of differentiation of cells in epithelial structures. Specifically, the vitamin is shown to inhibit the development of a keratinized squamous epithelium and permits differentiation into the secretory membrane of a totally different type. The target cell in this reaction would seem to be the basal cell of the stratum germinativum. The authors call attention to the fact that S. B. Wolbach and P. R. Howe (*J. Exp. Med.* **42,** *753 (1925)*) described the formation of a keratinizing layer of cells beneath the mucous-secreting ciliated epithelium of the nares and trachea in vitamin A-deficient rats, a change wholly comparable to that induced in tissue cultures of chick ectoderm by transfer from culture in a medium containing an excess of vitamin A to a normal medium.

LIVER RIBONUCLEIC ACID IN PREGNANCY

In a study of the biochemical changes of the rat liver in pregnancy R. M. Campbell and H. W. Kosterlitz (*J. Endocrinol.* **6,** *171 (1949)*) found several significant deviations from normal. A loss of liver glycogen with a rise in nonglycogen water was a usual finding. There was a slight rise in liver desoxyribonucleic acid (DNA). A much larger increase in ribonucleic acid (RNA) occurred which was independent of the changes taking place in total liver protein and phospholipids. The authors expressed the changes in RNA in terms of DNA with the assumption that this latter component, being found almost exclusively in the nuclei, is proportional to the number of liver cells.

The RNA content of the liver of nonpregnant rats was found to be 4.12 mg. per milligram of DNA. On the eighteenth to twentieth day of pregnancy RNA had risen to 6.04 mg. per milligram of DNA. A return toward control values was found by the fifth day of lactation (5.04 mg. per milligram of DNA). The increase in liver RNA was evident in both pregnant rats fed the stock diet and those fed a low protein diet for five days, when compared to nonpregnant rats fed a similar diet. The turnover rate of RNA phosphorus determined with P^{32} was not definitely increased. However, in view of the increased amount of RNA present, the total turnover was greater in the liver RNA from pregnant rats. When the fetuses were removed in pregnancy without removal of the placentas, the RNA content of the liver was still found to be higher than

in control rats. The removal of the placentas as well as the fetuses blocked the formation of excess RNA.

These observations have recently been extended by the same workers (R. M. Campbell and H. W. Kosterlitz, *J. Endocrinol.* **9**, *45 (1953)*) who present observations on pregnant mice, guinea pigs and cats. The findings in the mouse are very similar to those in the rat. In the livers of pregnant mice there was an increase in DNA when plotted against body weight minus uterus, but none when plotted against total body weight. The RNA content, on the other hand, was significantly increased when plotted against several indices. The effect of protein intake on liver protein and RNA content in livers from pregnant and control mice was studied by feeding a diet of sugar and fat supplemented with varying amounts of casein, from 0 to 40 per cent of the diet by weight. An increase in liver RNA and liver protein of both pregnant and control mice occurred with increasing dietary protein. A straight-line relationship existed between liver RNA and liver protein, both expressed in terms of DNA. The slope for both pregnant and non-pregnant mice was similar, but the RNA values for the livers of pregnant mice with a given liver protein content were always significantly higher than the controls.

The livers of pregnant guinea pigs showed no changes in DNA. An increase in liver RNA in pregnant guinea pigs was found, but quantitatively the increase was less than that found in rats and mice. Similar studies of cats showed that there was no elevation of liver RNA in pregnancy. The authors point out the difficulty in obtaining representative control values for cats.

Two possible explanations for the species differences in liver RNA content in pregnancy were suggested. First, the rate of fetal growth is proportionately greater in rats and mice than in the cat, which may indicate increased placental activity. Second, the placentas of the rat and guinea pig are of the type in which maternal blood is in contact with the chorion of the embryo, and might permit maternal absorption of a RNA-stimulating factor from the fetal placenta. In the placenta of the cat, the maternal endothelium is in contact with the chorion, and may be impervious to such a factor.

In a subsequent paper an attempt has been made to analyze the factors responsible for the excess RNA found in the livers of pregnant rats (R. M. Campbell, I. R. Innes, and H. W. Kosterlitz, *J. Endocrinol.* **9**, *52 (1953)*). Removal of the fetuses on the fourteenth day of gestation did not prevent the persistence of the placentas, but some decrease in the weight of these organs did occur. The RNA found in the liver after twenty-one days of gestation following removal of the fetuses on the fourteenth day was less than in control pregnant rats. The decrease in RNA seemed to be about proportional to the decrease in placental mass. When the ovaries as well as the fetuses were removed on the fourteenth day of gestation there was no significant difference in liver RNA content as compared to the rats with only the fetuses removed. The weights of the placentas were found, unexpectedly, to be greater after removal of the ovaries as well as the fetuses.

In addition to investigating the influence of the ovaries, a study was made of the effect of the adrenal and pituitary on the liver RNA of pregnant rats. Adrenalectomy, either alone or in combination with removal of the fetuses and/or ovaries, did not have a significant influence on liver RNA. An additional lowering of RNA was observed after hypophysectomy under a number of experimental conditions. Because of the poor food intake of the hypophysectomized rats the possibility that the changes in RNA might be due to low protein intake was considered. It was found that the decrease in RNA following hypophysectomy was greater than could be accounted for on the basis of a loss in liver protein. Pair feeding hypophysectomized and control rats indicated

that the change in RNA was not related to protein intake.

In explaining the excess RNA in the livers of pregnant rats two factors were considered important. The first is an unknown factor secreted by the placenta which does not require the presence of the fetus. The second factor is the presence of an intact anterior lobe of the pituitary. The pituitary hormones involved in producing excess RNA in pregnancy were not identified. Preliminary experiments with growth hormone suggest that this hormone is without effect on RNA. The authors suggest that the pituitary induces excess RNA by stimulating the production of estrogens. Administration of estrogens was effective in increasing liver RNA. In view of the failure of ovariectomy to prevent the formation of excess RNA, the importance of gonadotropins acting on the ovary does not seem great.

The metabolic significance of the changes in RNA of the liver in pregnancy remains to be determined. The relationship between liver protein content and RNA suggests some role of RNA in protein synthesis, but more fundamental information is needed.

EFFECT OF DIET ON CARBOHYDRATE ABSORPTION

Numerous efforts have been made during the last fifty years to discover the mechanism underlying the selective absorption of carbohydrates from the small intestine. While the physiologist of fifty years ago spoke of "vital activity," the biochemist today is likely to think in terms of phosphorylation. Both concepts attempt to explain why sugars such as glucose are absorbed much more rapidly than the smaller, more easily diffusible pentoses, why glucose and galactose are absorbed more rapidly than fructose, and why glucose can be absorbed against a concentration gradient, to mention but a few of the many perplexing aspects of carbohydrate absorption.

A recent paper by C. Long (*Biochem. J.* **53,** *7 (1953)*) on the effect of diet on hexokinase activity of the intestinal mucosa is the first to appear in some time which promises to shed new light on the absorption mechanism. Rats were maintained on basal, high carbohydrate, high fat, or high protein diets. The hexokinase content of the mucosa was increased by the high carbohydrate diet above that level observed on the basal ration. On the other hand, the high fat diet depressed the hexokinase content. The high protein diet caused no change.

Earlier work in other laboratories has shown that feeding a high carbohydrate diet will increase the rate of carbohydrate absorption while fasting, or the feeding of a high fat diet will decrease carbohydrate assimilation (R. G. Sinclair and R. J. Fassina, *J. Biol. Chem.* **141,** *509 (1941)*; E. M. MacKay and H. C. Bergman, *J. Nutrition* **6,** *515 (1933)*; S. Donhoffer, *Pflügers Arch. f. d. ges. Physiol.* **246,** *92 (1942)*).

Of great interest in this regard was the report of H. G. K. Westenbrink (*Arch. neerl. de Physiol.* **19,** *563 (1934)*) on the effect of dietary carbohydrate on the absorption rates of glucose, galactose and fructose. A diet rich in galactose enhanced the subsequent absorption of galactose and glucose but not of fructose. A diet high in fructose increased the absorption of fructose and glucose but not of galactose. Finally, a diet rich in glucose facilitated glucose absorption, but had no effect on the assimilation of galactose or fructose.

From the reports of L. Laszt and H. Süllmann (*Biochem. Z.* **278,** *401 (1935)*), E. Lundsgaard (*Z. f. physiol. Chem.* **261,** *193 (1939)*), F. Verzár and Süllmann (*Biochem. Z.* **289,** *323 (1937)*) and others it has become clear that the absorption of the rapidly assimilated sugars is accompanied by the accumulation of hexosephosphates in the

intestinal mucosa. Consequently there arose the popular concept that the absorption of such sugars as glucose, galactose and fructose is facilitated by phosphorylation. In its simplest terms this concept holds that the passage of any substance from the intestinal lumen into the mucosal cells leads to a significant accumulation of the absorbed substance in these cells, thus reducing the concentration gradient across the cell membrane and diminishing the driving force of the diffusion process. If, however, the absorbed substance can be transformed (through a process such as phosphorylation), a high concentration gradient, and thus the driving force, can be maintained. Evidence for this mechanism has remained largely circumstantial.

Demonstration of the accumulation of hexosephosphates during glucose absorption has led to extensive studies on the possible role of phosphatases in this process. The work of Long furnishes additional evidence that the role of the phosphatases must be a secondary one, that of the dephosphorylation of the accumulated hexosephosphates prior to the passage of free glucose into the blood stream. If phosphorylation is indeed the active cellular process, the "vital activity," the hexokinases must be primarily concerned. Indeed, the work of Long shows dietary effects on mucosal hexokinase content which parallel the well-known dietary effects on the absorption rate of glucose. While this demonstration strengthens the phosphorylation concept, it raises the question of how dietary manipulation alters the hexokinase content of the intestinal mucosa.

It is interesting to recall the effects of diet on other aspects of carbohydrate metabolism: glucose tolerance, glycogen synthesis and insulin content of the pancreas. All are similarly affected by diet. Fasting or feeding a high fat diet lowers glucose tolerance and glycogen synthesis and reduces the insulin content of the pancreas. Glucose absorption is retarded and, according to Long, the hexokinase content of the intestinal mucosa is reduced. In general, high carbohydrate diets have the opposite effects on these aspects of carbohydrate metabolism. It is difficult to believe that diet exerts these effects through several distinct mechanisms. Perhaps the primary effect of diet is on insulin production and secretion, while the other effects are secondary, perhaps through hexokinase.

MEGALOBLASTIC ANEMIA AND INFECTIONS

The occurrence of megaloblastic anemia in infancy, its frequent relationship with ascorbic acid deficiency, and its response to therapy with vitamin B_{12}, folacin and ascorbic acid have been the subject of previous reviews (*Nutrition Reviews* **4,** *338 (1946)*; **8,** *260 (1950)*; **9,** *52 (1952)*). The megaloblastic changes in the bone marrow of monkeys during the course of naturally acquired respiratory infections observed by C. D. May, C. T. Stewart, A. Hamilton, and R. J. Salmon (*Am. J. Dis. Child.* **84,** *718 (1952)*) suggested a study of the role of infection in inducing this anemia in monkeys.

The sterile abscesses which follow the intramuscular injection of turpentine proved to be a suitable experimental infection. Five animals were placed on milk diets supplemented with ascorbic acid, the fat-soluble vitamins, and members of the B-complex with the exception of folacin. Turpentine injections were given as often as necessary to maintain a suppurative process. A megaloblastic metamorphosis in the bone marrow occurred after a variable period of thirty-two to seventy-three days and was accompanied by anemia and leukopenia. Treatment with vitamin B_{12} administered intramuscularly was ineffective. Intramuscular doses of folacin of 15 mg. daily for two

days were rapidly effective. In one animal a concentrate of pteroylheptaglutamate equivalent to 100 micrograms of folacin by microbiologic assay was given orally for a twelve-day period without effect. In another, a spontaneous remission occurred when the abscess was allowed to heal.

Analysis of the livers of 3 animals showed normal ascorbic acid and vitamin B_{12} concentrations. The folacin and citrovorum factor contents were reduced to the level found in animals developing megaloblastic anemia as a result of ascorbic acid deficiency.

These observations demonstrate the ease with which megaloblastic anemia may be produced in the monkey fed a milk diet. Such diets supply amounts of folacin adequate during health, but the development of scurvy, respiratory infections, or turpentine abscesses is followed by megaloblastic anemia. Since this anemia responds specifically to therapy with folacin it is reasonable to suppose that the diet used in these experiments did not contain amounts of this vitamin sufficient to meet the presumably increased requirements following the experimental infections. The efficacy of small amounts of orally administered folacin in producing remissions in megaloblastic anemia in infancy has been noted (C. W. Woodruff, J. C. Peterson, and W. J. Darby, *Proc. Soc. Exp. Biol. Med.* **77,** *16 (1951)*). The failure of a small amount of the heptaglutamic conjugate of this vitamin to produce a remission in these experiments emphasizes the need for more quantitative information concerning the amounts of orally administered folacin required to reverse the megaloblastic anemia occurring in monkeys fed milk diets.

May and co-workers have classified infants showing megaloblastic bone marrows into three broad groups: those associated with ascorbic acid deficiency which may respond to treatment with ascorbic acid alone; those occurring following infections, many of which do not respond to vitamin B_{12}; and lastly a group responding initially to this vitamin. Examples of all three types occurring in infants are presented. The experimental analogues of the first two types have been studied in milk-fed monkeys by these authors.

The factors related to the response of certain cases to vitamin B_{12} remain obscure. Since the availability to the infant of the vitamin B_{12} in milk and its quantitative relationship to his requirement for this vitamin are unknown, the hypothesis that sick infants may not absorb or utilize vitamin B_{12} normally rests on theoretic grounds. Until more exact knowledge concerning the absorption and utilization of folacin by the human is available and indisputable evidence for a dietary requirement for folacin by man is presented, our concepts of the pathogenesis of megaloblastic anemia in infancy will remain incomplete. The conclusion of W. W. Zuelzer and F. N. Ogden (*Am. J. Dis. Child.* **71,** *211 (1946)*; *Nutrition Reviews* **4,** *338 (1946)*) that megaloblastic anemia in infancy is essentially a folacin deficiency has been amply confirmed. The experimental production of an almost identical syndrome in the monkey fed milk formulas commonly used in infant feeding by May and his co-workers has emphasized the role of factors other than a simple dietary deficiency of this vitamin in its pathogenesis.

THE NATURE OF THE XANTHINE OXIDASE FACTOR

The maintenance of a normal level of liver xanthine oxidase in the rat has been shown to be dependent on adequate nutrition. It has been found that the enzyme is reduced in concentration when the diet contains inadequate protein (*Nutrition Reviews* **11,** *23 (1953)*). More recently it has been shown that both essential and nonessential amino

acids are necessary for maintenance of a normal liver xanthine oxidase (G. Litwack, J. N. Williams, and C. A. Elvehjem, *J. Biol. Chem.* **201,** *261 (1953)*). W. W. Westerfeld and D. A. Richert (*Ibid.* **192,** *35, 49 (1951)*; *Nutrition Reviews* **10,** *74 (1952)*) have shown that a nutritional factor in addition to protein is necessary for the elaboration of normal amounts of rat liver and intestinal xanthine oxidase. This factor was found to be present in liver, and the addition of 10 per cent liver residue to the purified diet resulted in liver and intestinal xanthine oxidase activities comparable to those obtained by feeding a diet of natural foodstuffs.

The chemical nature of the active principle of liver residue has been elucidated by E. C. De Renzo *et al.* (*J. Am. Chem. Soc.* **75,** *753 (1953)*). These workers subjected liver residue to chemical fractionation procedures and assayed the fractions for the xanthine oxidase factor by the procedure described by Richert and Westerfeld (*loc. cit.*). In their assay procedure weanling rats are given a basal purified diet containing 21 per cent casein. The supplements to be assayed are added to this diet at the expense of glucose and after a two-week feeding period intestinal xanthine oxidase is determined. The addition of 15 per cent liver residue to this basal diet increased intestinal xanthine oxidase from 6 to 33 microliters of oxygen per twenty minutes per 283 mg. of tissue.

De Renzo *et al.* found that the xanthine oxidase factor could be partially liberated by autoclaving in water, and when thus liberated was dialyzable and stable to severe acid or alkaline treatment. The activity of the liver residue was found to be unaltered by ashing. These observations indicated that the active material was inorganic and the ash was assayed spectrographically. Many elements were present and further investigations were conducted by adding single salt supplements to the assay diet of Westerfeld and Richert. It was found that sodium molybdate would replace liver residue in maintaining rat intestinal xanthine oxidase. The addition of 1 mg. of sodium molybdate per kilogram of basal diet resulted in intestinal xanthine oxidase values equal to those obtained by the feeding of 10 per cent liver residue. The authors state that preliminary studies indicate that no other element is able to replace molybdenum in producing this effect. It will be of interest to learn if molybdenum is a constituent of the xanthine oxidase enzyme or is indirectly concerned in its elaboration. Regardless of the mechanism of the effect the results are of great interest to the experimental nutritionist in that they indicate a biochemical role for another dietary component.

DYES IN DIETARY CARCINOGENESIS

The problem of the influence of dietary constituents upon tumor formation and growth has occupied a large share of the efforts of oncologists (cancer specialists). In addition to the common nutrients and dietary factors, the non-nutritional substances occurring in food have been objects of study. A recent example of the latter type of investigation is a preliminary study of the possible carcinogenicity of dyes (R. Willheim and A. C. Ivy, *Gastroenterology* **23,** *1 (1953)*). These workers have attempted to determine whether certain dyes might act as external carcinogens by direct effect upon the tissues of the gastrointestinal tract when fed as part of the diet. Although they recognize the presence of innumerable substances in food which could be used in such a study they chose to use dyes because certain ones have been reported to be carcinogenic and because their presence might be easily detected in mucosal cells.

Using Wistar strain rats they employed 27 different dyes mixed with the diet in concentrations of 4 per cent by weight. Fourteen of the dyes were azo dyes, 6 were triphenylmethane types, 5 were xanthene types, and the remaining 2 were methylene blue and hematoxylin. In order to determine the ability of the dyes to stain the mucosa some animals in each group of 10 were killed after three weeks. Some were given the dye until death, while in others it was withdrawn from the diet a few days before examination. After the various parts of the gastrointestinal tract were rinsed and examined grossly for dye staining, the tissue was examined histologically.

Most of the dyes stained some portion of the gastrointestinal tract to some extent, but in most instances the forestomach of the rats was either stained very slightly or not at all while the glandular stomach was a very frequent site of staining. Sudan Red II, Sudan III, Sudan IV, methylene blue and tartrazine, however, stained the forestomach as much as or more than the glandular stomach. The small intestine and colon were stained less frequently, although they were more frequently and intensely stained than was the glandular stomach when pyronine, rhodamine B and phloxine were used. In most cases the dye disappeared grossly from the mucosa when it was withheld from the diet for one day, but it could still be detected in the gastric secretions. Aside from some swelling of the gastric rugae and occasional hyperplasia of the intestinal lymph follicles no other gross changes were observed.

Microscopic examination revealed granular deposits in many of the dye-stained tissues, such deposits being found mainly in the upper portion of the gland cells. Round cell infiltration occurred, vacuolated cytoplasm was observed in the dyed areas, and pyronine-methyl green histologic stains of the sections yielded suggestive evidence of decreased nucleic acid. This type of finding the authors interpret as indicative of altered cell structure and impaired physiology when there are dye deposits. Post mortem painting of the tissues with the dyes revealed some superficial granular deposits, thus suggesting that simple diffusion may play a part in the staining phenomenon.

Tumors which histologically resembled malignant lymphomas were observed in 7 animals receiving five different dyes. Five were in the abdominal lymph nodes, one was in the mediastinum and one on the forehead. The dyes incriminated in the development of these lesions were amaranth, ponceau SX, Guinea green B, light green SF yellowish, and hematoxylin, each producing one or two tumors. The earliest noted tumor was found in an animal fed hematoxylin for six months, while the majority of tumors were noted after eighteen to twenty months of dye ingestion. Three tumors showed intense dye staining in the central portion of cross-sections. In contrast to this incidence of 7 tumors in 33 animals receiving dyes, no tumors were seen in 50 control animals.

Another finding of some interest in the dye-fed animals was the occurrence in 5 rats of cirrhosis of the liver without strict evidence of neoplasia. These animals were receiving ponceau ✱3R, erythrosin, or Sudan IV, and as with the tumors, there was no incidence of cirrhosis in the control group.

This type of investigation is admittedly preliminary and susceptible to many refinements before its results can be considered conclusive. No implication of dyes in carcinogenesis as they ordinarily are used in foods should be read into these studies, however, for the authors have not hinted at this in their study and it is eminently clear that 4 per cent concentrations in the food far exceed the ordinary amounts in human diets. This study, nevertheless, appears to be worthy of further exploration, particularly in view of the possible information it may shed not only upon the genesis of lymphomas, but upon the occurrence of cirrhosis.

SOYIN, A PROTEIN IN RAW SOYBEANS

Soyin is a protein recently isolated from defatted soybean flour by I. E. Liener and M. J. Pallansch (*J. Biol. Chem.* **197,** *29 (1952)*) that has certain toxic properties. This protein is inactivated by heat treatment of soybeans. This observation has cleared some of the differing opinions regarding the unique effect of heat in improving the nutritive value of soybean protein, as first demonstrated by T. B. Osborne and L. B. Mendel (*Ibid.* **32,** *369 (1917)*).

In a recent publication, I. E. Liener (*J. Nutrition* **49,** *527 (1953)*) ascertained the extent to which soyin was responsible for the poor nutritive value of unheated soybean oil meal. The soyin used in rat-feeding trials was isolated from defatted soybean flour by the following procedure: Fifteen hundred grams of the material was suspended in 18 liters of distilled water. With constant stirring, the pH was adjusted to 6.7. After an hour the pH was brought to 4.6 with hydrochloric acid and the formed curd was allowed to settle overnight at refrigerator temperature. Most of the water extract was removed by siphon and the remainder was removed by filtration with the aid of suction. To each 10 liters of water extract were gradually added, with stirring, 3 kg. of ammonium sulfate. The resulting precipitate was separated by centrifugation, taken up in water and dialyzed against distilled water for thirty-six hours at refrigerator temperature. After removal of insoluble precipitates that formed during dialysis, the extract was adjusted to pH 4.6 with hydrochloric acid. After reprecipitation with ammonium sulfate, the active material was taken up in phosphate buffer (pH 6.1) and dialyzed against 60 per cent ethanol for twenty-four hours at -5 to $-10°$ C. The precipitate which formed in the dialysis bag was separated by centrifugation, taken up in water, and again dialyzed against cold distilled water. After any insoluble material was centrifuged off, the final solution was dried *in vacuo* from the frozen state.

On the basis of electrophoretic measurements, the final preparation contained about 78 per cent of the toxic protein soyin. The preparation contained very little antitryptic activity, but had marked hemagglutinating action. The earlier preparation reported in the paper by Liener and Pallansch (*loc. cit.*) was found to be devoid of either urease or antitryptic activity.

Weanling male rats were used for the feeding trials. The ration contained the following in per cent: raw or autoclaved defatted soybean flour, or casein, 25; salt mixture, 4; corn oil, 6; and sucrose, 65. It was supplemented with a complete vitamin mixture. When soybean flour served as the protein, the ration was modified by the addition of 0.6 per cent of DL-methionine at the expense of an equal amount of sucrose.

Using the growth supported by autoclaved soybean flour as a reference, unheated soybean flour produced a growth inhibition of about 50 per cent (43.8 g. body weight gain for the former and 20.0 g. for the latter for a two-week period). When the soyin preparation was added to the autoclaved soybean ration at a level of 1 per cent, body weight gain was decreased from 64 g. to 45 g. Significant, although less marked, growth decline was also demonstrated when this level of soyin was added to the diet containing casein as the protein source. The 1 per cent level of the toxic protein preparation in the diet was calculated to supply about the same amount of toxic material as would be present in a ration containing 25 per cent of raw soybean flour.

These results established that about half of the growth depression seen on the raw soybean diets could be attributed to the soyin present. The addition of 5 per cent of trypsin to the raw soybean diet increased the two-week gains from 24 g. to 49 g., whereas the autoclaved soybean diet per-

mitted gains of 66 g. This result seemed to support the view that about one-half the growth depression resulting from raw soybean flour feeding could be attributed to the presence of factors counteracted by trypsin, whereas the other half was attributable to the presence of soyin, as already mentioned. Further studies by Liener in which the paired-feeding technic was employed revealed that the growth-inhibiting effects of soyin were caused by a lowering of the food intake.

A potent soybean trypsin inhibitor, prepared according to the method of A. A. Klose, B. Hill, and H. L. Fevold (*Proc. Soc. Exp. Biol. Med.* **62,** *10 (1946)*), was also fed to rats and found to inhibit growth when included in the diet at a 2 per cent level. A 1 per cent level failed to depress growth, yet it contained considerably more antitryptic activity than was present in the soyin preparations, thus ruling out the possibility that the growth-inhibiting action of the soyin preparation was due to antitrypsin.

The isolation of the toxic protein soyin by Liener and Pallansch and its demonstrated growth-inhibiting effects are of interest in view of the observations that a number of legume proteins which did not contain a trypsin inhibitor (jack bean, lentil, and horse bean) were improved by heat treatment (*Nutrition Reviews* **9,** *28 (1951)*). Liener's results confirm the view that the growth-depressing effects of raw soybeans are due to two factors, only one of which can be overcome with crude trypsin.

It would seem logical to assume that the partial correction of growth inhibition with trypsin on raw soybean diets, as demonstrated by these studies, was due to overcoming the antitryptic activity of the raw meal. R. Borchers and C. W. Ackerson (*Proc. Soc. Exp. Biol. Med.* **78,** *81 (1951)*), however, showed that crude trypsin preparations could be autoclaved with live steam at 15 pounds pressure for thirty minutes and still counteract the growth-depressing effects of raw soybean oil meal. This heat treatment was shown to destroy all trypsin activity of the preparation. The mechanism by which crude trypsin exerts an improvement in growth in rats when it is added to a raw soybean diet, therefore, still remains obscure.

NOTES

Fatty Acid Contents of Selected Foods Before and After Cooking

The essential quality of certain unsaturated fatty acids in animal nutrition has long been known; however, the essential fatty acid content of foods has not been extensively investigated. I. C. L. Chang and B. M. Watts (*J. Am. Oil Chem. Soc.* **29,** *334 (1952)*) have prepared a survey of this subject with a report on meat and poultry before and after cooking.

Spectrophotometric methods permit reasonable speed and accuracy for these analyses of individual acid contents. Samples of pork, lamb and beef, both fresh and frozen for six to eight months at 10° F., were from animals of known dietary regimens. Poultry was from a local market.

Only traces of peroxides were found in any fresh meats or cured pork. They developed during roasting of large cuts, being higher in the drippings than in the meat itself. Pork drippings had a rancid odor. Pork products developed considerable peroxide during the frozen storage. Cooking decreased this content, especially in the drippings.

Fatty acid distribution in fat from raw meat varied greatly with the species. Linoleic acid content was 1 to 2 per cent in beef and lamb fat and 20 to 30 per cent in turkey and chicken fat. Pork fat was intermediate between these two groups. Variations in linolenic and arachidonic acids were not so marked as those in linoleic acid, but poultry fats were generally higher in polyunsaturated acids than the animal fats. In addition, spectroscopic data indicated the presence of small amounts of acids with pentaene and hexaene conjugation in poultry fats, especially turkey. Various regions of the same animal showed significant differences in acid distribution.

In all samples, with the exception of roast chicken, there was a loss of total unsaturated fat during cooking. This loss exceeded 2 per cent only in the case of roasted pork loin and leg of lamb, which showed considerable peroxidation during cooking. These losses were apparently mostly in oleic acid. Chicken fried in vegetable oil and bacon as eaten were highest and lamb and beef the lowest in linoleic acid among all meats. Vegetable oil contributed much to the high value for fried chicken. Linolenic and arachidonic acid contents of all meats studied were small in comparison with linoleic acid. Therefore variations in these acids will not greatly affect the nutritive value of meats as sources of essential fatty acids. Turkey skin is particularly high in linoleic acid. The data presented in tabular form may well be of value for human dietary studies. Common methods of cooking do not appreciably change the fatty acid composition of the fats of meat and poultry.

Another study by I. C. L. Chang, L. I. Y. Tchen, and B. M. Watts (*J. Am. Oil Chem. Soc.* **29,** *378 (1952)*) dealt with fatty acid changes in commercial food fats, when used in baked products and hard candy and as a medium for deep fat frying. These uses involve temperatures much higher than 100° C.

The fat recovered from various baked products (pie crust, ginger snaps and biscuit, all made with plain lard, the least stable of the usual shortening materials) did not differ significantly in its fatty acid composition from the original fat used in the dough. Ordinary baking procedures thus cause little loss of unsaturated acids. Butterfat, extracted from butterscotch candy, showed a small loss of total unsaturation, mostly in oleic acid. Soybeans, soaked overnight and simmered in water for three and one-half hours, showed little change, indicating no oxidative action on fatty acids during this treatment.

The peroxide value of lard increased to a maximum when lard was heated to 180° C. for ten minutes. After forty minutes it decreased to a negligible value, indicating that peroxides were decomposed as fast as formed.

In corn oil, on the other hand, peroxides reached a constant value after forty minutes heating and remained constant during four hours. Standing overnight increased the value, but it decreased to the same equilibrium value after thirty minutes additional heating.

Changes in fatty acid composition of lard during the fifty minutes of heating were very small except that conjugated diene increased slightly. In corn oil prolonged heating increased conjugated dienes three-fold, accompanied by a decrease in iodine number. Changes in oleic acid content were small, and linoleic acid was definitely decreased. The small changes are explained by slight oxidation of the large amount of linoleic acid present. Oxidation of linolenic acid was not great.

It appears then that only minor changes occur in fatty acid composition of fats in the ordinary cooking procedures studied. Peroxides form somewhat in relation to the temperature and time of cooking, but may be rapidly decomposed by heat.

Nutritional Investigation of Synthetic Vitamin A in Margarine

Margarine, fortified as it is with at least 15,000 U.S.P. units of vitamin A per pound, is an important food source of vitamin A. Synthetic vitamin A concentrates are now readily available and their blandness and high concentration obviate objectionable flavors and odors in the margarine.

The nutritional effects of synthetic vitamin A palmitate in very high concentrations in margarine were studied by S. R. Ames, M. I. Ludwig, W. J. Swanson, and P. L. Harris (*J. Am. Oil Chem. Soc.* **29**, *151 (1952)*), who maintained three generations of rats on adequate diets containing 7.5 per cent margarine oil, one with natural vitamin A ester at the usual level (18,500 U.S.P. units per gram) and the other with synthetic vitamin A palmitate at a level one hundred times as high.

The criteria of response were growth rate, reproductive performance, and lactation ability. Fresh diets, prepared weekly and stored in a refrigerator, were fed ad libitum. No statistical differences were found in average weights of either male or female rats on the two diets. These measurements were made throughout a twenty-six-week period after weaning. As for reproductive performance, comparable fertility and mean size of litters were obtained with female rats fed these two diets. As the principal criteria of lactation, the body weights of the young at time of weaning were comparable for the two groups. In general appearance, such as fur condition, no differences were found. Ten rats, 5 from each group, were submitted to a neutral observer for examination. Gross observations at necropsy showed them to be in good physical condition with no unusual changes. Histologic studies on these rats indicated no marked differences in tissue and organs of animals maintained on the two diets.

Thus it appears that excessive vitamin A consumption in the form of the synthetic palmitate in margarine is not harmful to the rat.

Recent Nutrition Book

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